**2806-Plat**
**Molecular Simulations of Mutually Exclusive Folding in a Two-Domain Protein Switch**
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A major challenge with testing designs of protein conformational switches is the need for experimental probes that enable the individual domains to be independently monitored. One way to circumvent this issue is to use a molecular simulation approach in which each domain can be directly observed. Here we report the first molecular simulations of mutually exclusive folding in an engineered two-domain protein switch, providing a detailed view of how folding of one protein drives unfolding of the other in a barnase-ubiquitin fusion protein. These simulations successfully capture the experimental effects of interdomain linker length and ligand binding on the extent of unfolding in the less stable domain. In addition, the effect of linker length on the potential for oligomerization, which eliminates switch activity, is in qualitative agreement with experiment. We also perform the first simulations exploring the ease of unfolding a protein via localized compression. Finally, we report the effect of linker length on rates of unfolding and refolding of each domain, providing novel kinetic insights on mutually exclusive folding. Our results demonstrate that molecular simulations can be useful virtual assays of switch activity that can aid the rational design of bi-functional switches.

**2807-Plat**
**Exploring the Trafficking, Ligand-Binding Activity, and Unfolding of a Model GPCR**
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G protein-coupled receptors (GPCRs) are integral membrane proteins vital for cellular signaling and constitute one of the major drug targets. Despite their importance, relatively little information regarding their structure, folding, and stability has been published.
This work describes the impact of disulfide bonds on the expression and structural stability of the human adenosine receptor, $A_{2A}$ ($hA_{2A}R$). The crystal structure of this receptor revealed four disulfide bonds present in extracellular loops that could contribute to expression, stability, or ligand binding or to a combination of these.
To test the role of these residues, cysteine to alanine mutants of $hA_{2A}R$ were created; expression and ligand-binding activity of the constructs were tested in mammalian (HEK293) and yeast (*Saccharomyces cerevisiae*) cells. Once purified from yeast, unfolding of the $hA_{2A}R$ through thermal and chemical means was monitored via intrinsic tryptophan fluorescence and circular dichroism. The effect of ligand addition and reduction of disulfide bonds was also investigated.
Thermal and chemical denaturation were not reversible, yet clear differences in the unfolding behavior were observed upon ligand binding via circular dichroism and fluorescence spectrometry. We found that the stability of $hA_{2A}R$ was increased upon incubation with the agonist N6-cyclohexyladenosine or the antagonist theophylline. When extracellular disulfide bonds were reduced with a chemical reducing agent, the ligand binding activity decreased by ~40%, but reduction of these bonds did not compromise the unfolding transition observed via urea denaturation. Overall, these approaches offer a general strategy for characterizing the effect of disulfide bonds and ligand effects on the stability of GPCRs.

**2808-Plat**
**Determinants of Cooperativity in Repeat Protein Folding**
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Repeat proteins have been popular model systems in protein folding research due to their modular architecture. Although each repeat has the same fold, because of sequence variation, the folding energy is not distributed evenly among the repeats. Therefore, it is challenging to quantify the repeat-repeat interactions and the intrinsic folding energies of every repeat. To overcome this difficulty, we designed a consensus ankyrin repeat(CANK), which is brought together to make arrays of identical repeats. By solution NMR, we solved the structure of our three repeat CANK, NRC, and verify that NRC adopts the canonical Ankyrin repeat protein fold. Our SAXS measurements show that CANKs in different length arrays also adopt the same fold. By fitting unfolding transitions of a limited number of constructs to Ising model, we found that the individual repeats have low intrinsic stability, but the interfacial interactions are highly stabilizing. We also extended our Ising formalism to include the temperature dependence of unfolding free energy and we quantified the enthalpic and entropic contribution to intrinsic and interfacial stabilities, as well as, the uneven distribution of heat capacity of unfolding into the intrinsic and interfacial components. Our results suggest that intrinsic folding resembles secondary structure formation(loss of conformational entropy) and interfacial folding involves hydrophobic desolvation(entropy increase and negative heat capacity change upon folding). To separate out the contribution of long range electrostatics to intrinsic and interfacial stability, we measured the salt dependence of CANK stabilities. Our results show that NaCl stabilizes the interfaces and has marginal destabilizing effect on intrinsic stability. Currently we are trying to identify the charge-charge interactions responsible for the high salt dependence of interfacial stability by NMR techniques and single point substitutions.

**2809-Plat**
**Outer Membrane Secretion Efficiency of Autotransporter Virulence Proteins Correlates with Passenger Domain Folding Properties**
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Autotransporters (ATs) are the largest class of extracellular virulence proteins secreted from Gram-negative bacteria. Each AT is synthesized as a tripartite pre-protein containing an N-terminal signal sequence that directs secretion across the inner membrane, a central ''passenger'' domain that becomes the mature extracellular virulence protein, and a C-terminal outer membrane (OM) porin domain that is essential for OM transport. AT passenger domains have highly diverse sequences, lengths, and functions, but almost all are predicted to contain β-helical structure. It was originally proposed that an AT protein autonomously catalyzes transport of its own passenger domain across the OM, but recent results have cast doubt on this model. Moreover, in the absence of a significant quantity of ATP or proton gradient across the OM, the driving force for efficient OM secretion remains unclear. Here we demonstrate a direct correlation between localized regions of AT passenger domain stability ($\Delta G_{unfolding}$) and OM secretion efficiency. Destabilizing the C-terminus of a passenger domain β-helix significantly reduced OM secretion efficiency. In contrast, destabilizing more N-terminal portions of the passenger domain produced a linearly correlated increase in OM secretion efficiency. Thus, C-terminal passenger domain stability facilitates OM secretion, whereas N-terminal stability hinders it. The contributions of regional passenger stability to OM secretion efficiency demonstrate a crucial role for the passenger domain itself in directing its secretion across the OM. These results indicate that the folding properties of distinct regions of the passenger domain provide a directed molecular driving force, for use as a generalized transporter device. The regionalized distribution of AT passenger domain stability provides a unique solution for the directed transport of macromolecules across biological membranes, defining a new category of ATP-independent Brownian motor.

## Platform AY: Calcium Signaling Pathways

**2810-Plat**
**Phospholipase C-Epsilon Couples cAMP Production and Epac2 Activation to the Facilitation of Calcium-Induced Calcium Release (CICR) in Pancreatic Beta Cells**
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We provide the first report that a novel phosphoinositide-specific phospholipase C-epsilon (PLC-epsilon) is expressed in the islets of Langerhans, and that the knockout (KO) of PLC-epsilon gene expression in mice disrupts the action of GLP-1 receptor agonist Exendin-4 to facilitate CICR in the beta cells of these mice. Thus, in the present study in which wild-type (WT) C57BL/6 mouse beta cells were loaded with the photolabile Ca2+ chelator NP-EGTA, the UV flash photolysis-catalyzed uncaging of Ca2+ generated CICR in only 9% of the beta cells tested, whereas CICR was generated in 82% of the beta cells pretreated with Exendin-4. This action of Exendin-4 to facilitate CICR was reproduced by cAMP analogs that activate PKA (6-Bnz-cAMP-AM) or Epac2 (8-pCPT-2'-O-Me-cAMP-AM) selectively. However, in beta cells of PLC-epsilon KO mice, and also Epac2 KO mice, these test substances exhibited differential efficacies in the CICR assay such that Exendin-4 was partially effective, 6-Bnz-cAMP-AM was fully effective, and 8-pCPT-2'-O-Me-cAMP-AM was without significant effect. Importantly, transduction of PLC-epsilon KO beta cells with recombinant PLC-epsilon rescued the action of 8-pCPT-2'-O-Me-cAMP-AM to facilitate CICR, whereas a K2150E